assign gene functions through the use of tissue-specific gene targeting (Gu et al., Science **265**:103-106 (1994); Kuhn et al., Science **269**:1427-1429 (1995); and Rajewsky et al., J. Clin. Invest. **96**:600-603 (1996)).

Conditional tissue-specific gene inactivation is accomplished through a binary transgenic mouse system, similar in principle to the one described above for conditional ablation of cell lineages. Here, the mating partner carrying the "activator" is derived from heterozygotic mutant ES cells containing a retroviral insertion in one of the two alleles of the gene to be subjected to the conditional tissue-specific inactivation. This mouse produces rtTA only in cells synthesizing the target gene (Figs. 3 and 4). the other mating partner, i.e., the one with the silent "weapon," carries a conditionally expressed ribozyme and a conditionally expressed recombinase.

Ribozymes are molecules capable of catalyzing sequence specific cleavage of targeted RNAs (Altman, Proc. Natl. Acad. Sci. USA **90**:10898-10900 (1993)). In this system, the ribozyme is preferably expressed using an RNA polymerase III (Pol III) dependent promoter, such as the U6 small nuclear RNA promoter (Das et al., EMBO J. **7**:503-512 (1988)). The Pol III promoter synthesizes the appropriate ribozyme only in the presence of rtTA and tetracycline derivatives. In addition, the constitutive Pol III promoter is preferably separated by transcription terminators from the ribozyme sequences. Each ribozyme is specifically designed to target and inactivate the gene of interest (according to published protocols, for example, by Altman, Proc. Natl. Acad. Sci. USA **90**:10898-10900 (1993); and Liu and Altman, Genes Dev. **9**:471-480 (1995)). The presence of the terminators blocks downstream transcription (Das et al., EMBO J. **7**:503-512 (1988)) and thus interferes with the synthesis of the ribozyme. The terminator sequences are flanked by FRT or loxP (i.e., the recognition sequence of either the *Saccharomyces cerevisiae* Flp recombinase (Dymecki, Proc. Natl. Acad. Sci. USA **93**:6191-6196 (1996)) or the bacteriophage P1 CRE recombinase (Sauer, Methods Enzymol. **225**:890-900 (1993))). Flp or CRE is expressed only in the presence of rtTA

and tetracycline derivatives.

In offspring containing both transgenes, Flp or CRE is produced in cells expressing the target gene when tetracycline derivatives are administered to the animal. Production of Flp or CRE leads to recombinational excision of the termination sequences and synthesis of the ribozyme in those cells. As a result, the target gene is subjected to ribozyme action, and the phenotype of this conditional tissue-specific gene inactivation event is amenable to analysis.

Another approach for conditional tissue-specific gene inactivation is based on conditional functional complementation between the disrupted and wild type alleles of the mouse gene or between the disrupted mouse gene and its wild type human homolog. This is a two step procedure that first involves mating of heterozygotic mice carrying the retroviral sequences of the present invention integrated in a particular gene to heterozygotic mice containing an extra copy of the wild type version of this gene under the rtTA-dependent promoter. Crossing F1 offspring containing both transgenes generate mice that are homozygotic in the disrupted gene but that also carry the wild type allele under the rtTA-dependent promoter. As a result, in the F2 mice, the wild type allele is expressed in the presence of tetracycline derivatives in the same cells that express the mutant gene. The presence of the wild type gene rescues the mutant phenotype which, in turn, may be assessed, when desired, upon withdrawal of the tetracycline derivatives. The very same approach may be used to complement the disrupted mouse gene with its human homolog, which is then expressed in the same cells that express the mouse mutant gene. If a human disease state gene is utilized in this technique, the F2 mice obtained may be used as animal models of the human disease, for example, to study the disease or isolate or identify therapeutic compounds.

Use of the MAGEKO process for conditional ectopic expression of the gene of interest in any desired tissue. Targeted gene expression is a powerful method for assigning function to genes, as has been demonstrated in several instances (Balling et al., Cell **58**:337-347

(1989); Kessel et al., Cell **61**:301-308 (1990); Brand and Perrimon, Development **118**:401-415 (1993); and Halder et al., Science **267**:1788-1792 (1995)). The retroviral vectors of the present invention are designed to utilize this powerful approach. According to this aspect of the invention, conditional targeted expression of a gene of interest is accomplished through a binary transgenic mouse system, similar to those described above. Again, in this system, one mating partner expresses rtTA under the control of the promoter associated with the gene having the retroviral insertion; as such, rtTA is synthesized only in cells expressing the mutant gene (Figs. 3 and 4). The other mating partner contains the gene of interest and synthesizes this gene product conditionally, i.e., only in the presence of both rtTA and tetracycline derivatives. In offspring having inherited both transgenes, the gene of interest is specifically expressed only in cells where the gene having the retroviral insertion is expressed, and only in the presence of tetracycline derivatives. The physiological consequences of this conditional targeted gene expression is thereby amenable to analysis in the offspring.

Importantly, this approach provides an unlimited number of different target tissues for analysis; in theory, every tissue in the animal can be selected, if desired, to study the consequences of the conditional ectopic expression of a gene of interest.

MAGEKO allows establishment of animals with conditional tumors in any desired cell type. The binary transgenic mouse system is also useful for the generation of animals with conditionally induced tumors. Here, one mating partner expresses rtTA under the control of the promoter of the gene with the retroviral insertion, and thus synthesizes rtTA only in cells expressing the mutant gene (Figs. 3 and 4). The other mating partner carries conditionally produced "neoplastic factors," such as combinations of oncogenes (Bishop, Cell **64**:235-248 (1991); and Hunter, Cell **64**:249-270 (1991)) and (if necessary) other facilitating genes, such as telomerase (deLange, Proc. Natl. Acad. Sci. USA **91**:2882-2885 (1994); Counter et al., Proc. Natl. Acad. Sci. USA **91**:2900-2904; and Sharma et al., Proc. Natl. Acad. Sci. USA **92**:12343-12346 (1995)). These factors are